LOW DOSE RADIATION RISK: A BIOLOGICAL REALITY CHECK

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ABSTRACT

All current radiation risk estimates and all radiation-protection standards and practices are based on the so-called "Linear No-Threshold Hypothesis". This paper summarizes results from some of our low dose and/or low dose rate experiments with low LET radiation in human and rodent cells, and in animals, and determines if the results support or reject the LNT hypothesis.

When DNA damage is created by radiation in a cell, there are three possible outcomes, an error-free repair which restores the cell to normal, cell death by apoptosis, or error prone repair that creates a mutation and cancer risk. The LNT hypothesis predicts that risk is influenced only by dose, and therefore that the relative proportions of these three biological possibilities must be constant. If they were not constant, then risk would vary with their relative proportions, i.e., not only as a function of dose. We have tested the influence of prior low doses and low dose rate exposures on these processes. As a measure of the overall effect of these processes, we measured the frequency at which rodent cells in tissue culture are transformed into cancer cells after a low dose exposure.

The results show that low dose radiation induces an increase in error-free DNA repair competence. That repair system increases the probability of correctly repairing either radiation-induced or spontaneous DNA damage, or of triggering cell death if the repair is incorrect. This response therefore reduces the overall risk of either radiation-induced or spontaneous transformation to malignancy. It is apparent from these experiments that biological variables are important in determining the consequences of radiation exposures and that the risk of DNA damage is neither constant nor additive nor increasing with dose.

We have reported the results of similar investigations in mice. In one experiment, low doses of *in vivo* β-irradiation of mouse skin 24 h prior to treatment with a DNA damaging chemical carcinogen reduced tumor frequency by about 5-fold. This result is consistent with the cell-based studies described above. It implies that the radiation exposure stimulated an error-free DNA repair system that was able to recognize and remove much of the chemically produced DNA damage. In another experiment, a prior low dose exposure delivered at low dose rate delayed the onset of myeloid leukemia induced in genetically normal mice by a subsequent exposure to a large dose. A similar result was seen in mice that were cancer prone due to a genetic defect, showing that low doses also protect mice predisposed to cancer.

The results indicate that low doses, or doses delivered at low dose rate, reduce rather than increase cancer risk in cells and in animals. The results contradict the LNT hypothesis.

INTRODUCTION

All current radiation risk estimates and all radiation-protection standards and practices are based on the "Linear No-Threshold Hypothesis" which states that risk is linearly proportional to dose, without a threshold. This hypothesis therefore predicts that:

- every dose, no matter how low, carries with it some risk
- risk per unit dose is constant, additive, and can only increase with dose
- biological variables are insignificant compared to dose

This paper presents the results of some of our low dose and/or low dose rate experiments with low LET radiation in human and rodent cells, and in animals, and determines if the results support or reject the LNT hypothesis as it applies to the risk of most concern, cancer. However, in order to properly compare the predictions of the LNT hypothesis with the actual experimental data, several biological and physical considerations must be kept in mind.

In considering radiation induced cancer, two biological points are important. First, cancer arises from changes in a single cell. This point is important because it defines the limits of the meaning of "low dose". Unlike the concept of whole body dose, where dose is averaged over all cells in the body, a single cell is the smallest volume that is relevant for carcinogenic risk. The lowest possible dose is, therefore, that dose which can be deposited in a single cell. However, cancer formation is a multi-step process. While the first step may be the direct result of the radiation exposure, some of the multiple changes required to produce a cancer will occur after the exposure, and their rate of occurrence defines the latent period, the time between exposure and the appearance of cancer. While one measure of risk is the frequency of cancer, another is the amount of lifespan lost, as determined by the latent period.

It is also important to recognize some physical characteristics of radiation:

- radiation deposits energy, and damage, in tracks
- the smallest dose a cell can receive is that deposited by a single track
- at total doses which are less than one track/cell, not all cells are hit, i.e., some cells receive no dose; however, those that are hit still receive the dose deposited by one track.

While the lowest possible dose to a cell is that deposited by one track, the actual dose depends on the nature of the radiation. For example, a single alpha particle track can deposit tens of cGy while a single ^{60}Co-γ ray will deposit, on average, about 1 mGy. The experiments presented here describe mainly gamma-ray exposure and therefore the minimum possible dose to a cell is about 1 mGy.

If we consider the potential biological outcomes of a radiation exposure to a normal cell, the first step depicted in Figure 1, there are three general biological outcomes of DNA damage as shown in Figure 1(1).

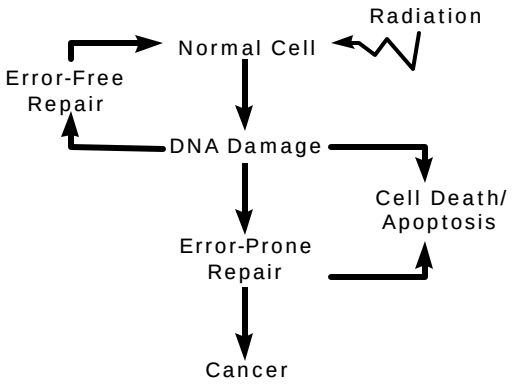

Fig. 1. Possible outcomes of a cellular radiation exposure in a normal cell.

When DNA damage is created by radiation in a cell, there are three possible outcomes. Two of those outcomes, an error-free repair which restores the cell to normal or cell death by apoptosis (programmed cell death) produce no risk of carcinogenesis since correctly repaired cells or dead cells do not produce cancer. Only the third possibility, an error prone repair that creates a mutation, results in cancer risk. The LNT hypothesis predicts that risk is influenced only by dose, and therefore that the relative proportions of these three biological possibilities must be constant. If they were not constant, then risk would vary with their relative proportions, i.e., not only as a function of dose.

EXPERIMENTAL RESULTS AND DISCUSSION

Cellular studies

Radiation exposure of cells can result in breakage of chromosomes, which indicate DNA double strand breaks, a type of damage which can result in the formation of such cancers as leukemia. The ability of cells to repair such lesions therefore reflects the cancer risk which ultimately results from this kind of radiation damage. The micronucleus (MN) assay can be used to measure the competence of the cells at repairing such breaks. Most radiation-induced MN contain unrepaired pieces of chromosomes. Counting the frequency of micronuclei after an exposure therefore provides a measure of the ability of cells to repair broken chromosomes (and therefore DNA double strand breaks) in response to radiation damage.

We have tested the influence of low doses and low dose rate exposures on the ability of human skin cells to repair radiation breaks in chromosomes (2). The LNT hypothesis predicts that the consequences of two doses would be additive. Figure 2 shows the MN frequency in cells exposed to a moderate dose (0.5 Gy) delivered at a low dose rate (2.5 mGy/min) and then immediately (0h) or after 5h, to a high dose (4 Gy) delivered at a high dose rate (1.8 Gy/min). The combined exposure resulted in less broken chromosomes than the single acute 4 Gy exposure alone, and when the doses were separated by a 5 h incubation, the resulting MN frequency was even less. This experiment indicates that the low dose rate exposure had stimulated the cells to increase their ability

to repair broken chromosomes, such that the consequences of the second large exposure were reduced. It is apparent from this experiment that biological variables are important in determining the consequences of radiation exposures and that the risk is not proportional to dose, results that do not support the LNT hypothesis.

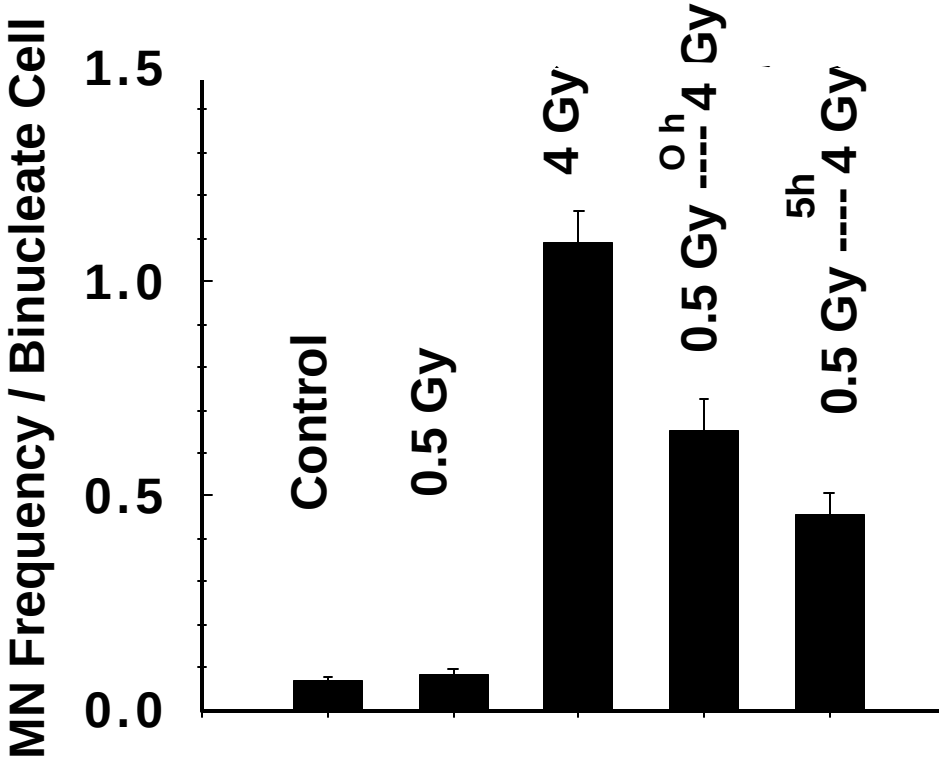

Fig. 2. Repair of broken chromosomes in human fibroblasts

Figure 4 shows that the same result occurs at 1 mGy, the lowest γ dose possible in a single cell since it represents, on average, a single track per cell (3). The figure also shows that higher doses, representing multiple tracks/cell, produce the same result as one track/cell when those tracks from the high doses are delivered at a low dose rate (3 mGy/min) i.e., spaced out in time. In all cases the cells were given 3h after the first (adapting) exposure to allow equivalent time for resistance to develop.

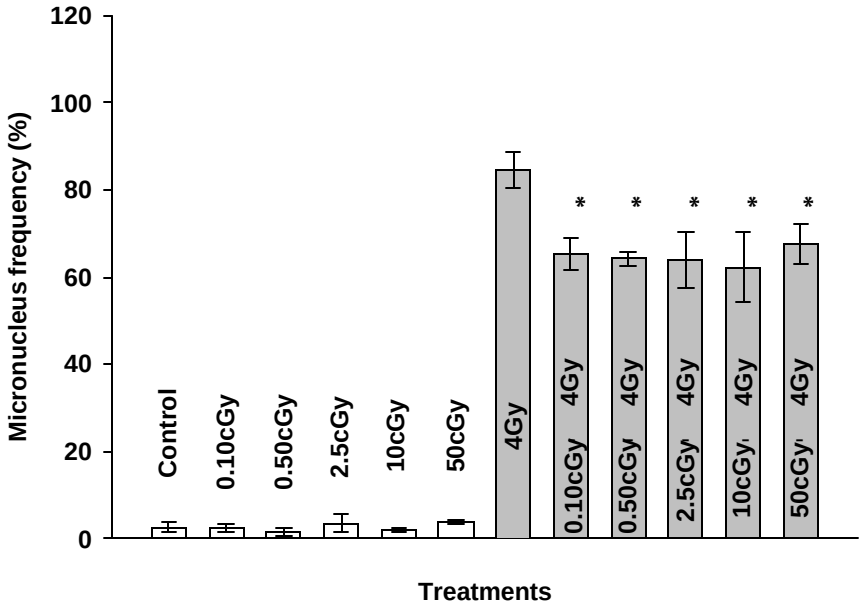

Fig. 3. Ability to repair broken chromosomes in cells adapted by exposure to low doses

While 1 mGy represents an average of one track per cell, radiation is a stochastic process and not all cells receive one track. Some cells receive two or more tracks, but the data in Figure 3 indicate that higher doses do not increase the response. Conversely, at 1 mGy many cells receive no hit at all, and yet the average response is not different from that at higher doses where all cells certainly receive multiple tracks. This data demonstrates, therefore, that cells which receive no hit, and therefore no dose, can still respond and increase their ability to repair broken chromosomes. This phenomenon is called a by-stander effect, where the response of a cell, which does receive a hit is transmitted, via cell to cell signal processes, to cells which have received no dose.

When we examined whether these radiation adapted cells in Figure 3 applied their increased repair competence uniformly to each chromosome, we found that the cells now displayed a bias in the repair of broken chromosomes (Figure 4) (4).

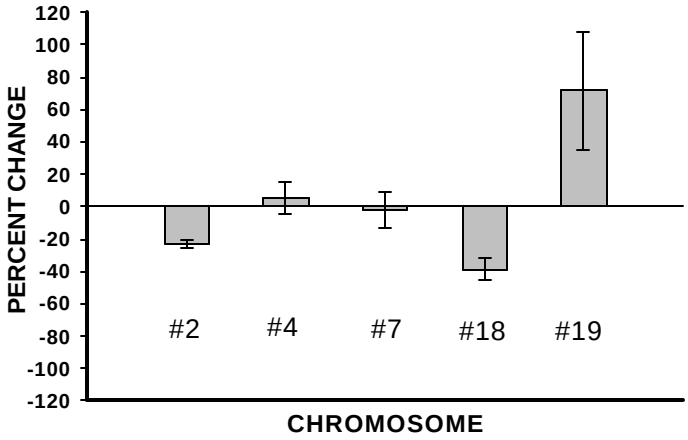

Fig. 4. Change in the frequency of broken chromosomes in micronuclei, which form after exposure of radiation adapted cells.

Less broken pieces of chromosomes 2 and 18 appeared in micronuclei after a 4 Gy exposure when cells were first given a prior 10 cGy dose, indicating that the adapted cells now preferentially repaired breaks in these chromosomes. In contrast, chromosome 19 was more frequently left unrepaired while repair of chromosomes 4 and 7 was unchanged . It appears, therefore, that low dose exposure to radiation altered the risk to some chromosomes and by implication, to the genes on those chromosomes. This result demonstrates that exposure of cells to a low dose changes not only the magnitude but also the very nature of the outcome. This response of cells indicates that the basic assumption of the LNT, that only the magnitude of the risk changes with dose, is incorrect.

This result also points out the difficulty in estimating the implications for risk of any measure of mutation in one specific gene or chromosome. A low dose may increase, decrease, or cause no change in repair of a specific chromosome and therefore of the genes on that chromosome. Consequently, translating that observation to the risk of cancer is not straight forward. In addition to the DNA repair being biased for or against some chromosomes or genes, the radiation-induced increase in repair competence could reflect either error-free repair, which would decrease risk, or error-prone repair, which would increase risk (Figure 1). Using an assay, which just measures rejoining of broken chromosomes does not distinguish between these possibilities.

The above experiments tested the predictions of the LNT hypothesis for two of the three possible outcomes (Figure 1) of a radiation exposure of a normal cell. The influence of a low dose on the third possibility, death by apoptosis, has also been tested. Comparing the extent of apoptosis induced in human lymphocytes by exposure to 2 Gy with the extent of apoptosis induced by 2 Gy in cells pre-exposed 6 h earlier to 10 cGy, the pre-exposed cells showed about a 20% increase in the number of apoptotic cells. This data is based on lymphocytes taken from 26 individuals (7-9). These results show that low doses amplify the probability of apoptotic cell death resulting from a second exposure. This sensitization of cells to radiation-induced cell death increases the probability that a cell will die rather than survive with a mutation, an outcome that is believed to reduce cancer risk in the whole organism.

While the data on low dose sensitization of cells to apoptosis, and the DNA repair data shown in Figures 2-4 do not appear to be consistent with some of the predictions of the LNT hypothesis, they do not directly test the hypothesis, since only DNA repair competence or bias, or cell death by apoptosis, but not cancer risk, was measured. As a direct test we used an assay that measures the frequency at which rodent cells in tissue culture are transformed into cancer cells. Some of the results are shown in Table I and provide a measure of changes in carcinogenic risk as a result of the low dose radiation exposures (5). The data show that a large (4 Gy) high dose rate (2 Gy/min) exposure increased the transformation frequency

Table I: Reduction in the risk of radiation-induced malignant transformation by a prior chronic exposure.

Treatment	**Transformation Frequency** ($\times 10^{-4}$)
Control	3.7
4 Gy (high dose rate)	41
100 mGy (low dose rate) + 4 Gy (high dose rate)	16

about 10-fold over the spontaneous frequency in these cells. However, a 100 mGy low dose rate exposure (2.4 mGy/min) immediately before the 4 Gy exposure did not further increase risk, as predicted by the LNT hypothesis, but actually decreased risk by 2- to 3-fold. This result is therefore contrary to current assumptions of cancer risk from multiple exposures, and suggests that low dose rate exposures are protective against subsequent exposure. The result is consistent with the concept that low doses stimulate cells to increase their capacity for an error free type of DNA repair (Figure 1) and the cells then selectively apply that repair to damage in those chromosomes and genes (Figure 4) which would otherwise create a risk of cancer formation.

While the data in Table I showed that the combined risk of the two exposures was less than that of the single exposure alone, it can also be seen that the net risk is still about 4-fold higher than the inherent spontaneous (non-radiation-induced) risk in the absence of radiation. The LNT hypothesis ultimately predicts that any dose, no matter how small, increases the risk of cancer. Using the rodent cell transformation assay, we directly tested that prediction, and the results are depicted in Table II (6). At an average of one track per cell (1 mGy) the risk of spontaneous transformation was reduced from that which occurred spontaneously in the

Table II: The influence of low doses delivered at low dose rate (2.4 mGy/min) on the risk of spontaneous malignant transformation.

Treatment	**Transformation Frequency** ($\times 10^{-3}$)
Control	1.8
1.0 mGy	0.62
10 mGy	0.39
100 mGy	0.49

absence of radiation exposure. The data also show that higher doses, up to 100 mGy delivered at a low dose rate, produced the same 3-4 fold reduction in spontaneous transformation risk. As noted above, at 1 mGy and an average of one track per cell, not all cells are hit, yet the overall response is not statistically different from many tracks per cell. This data therefore also shows that radiation-induced by-stander effects operate to reduce spontaneous cancer risk in cells, which do not actually receive a radiation exposure.

These DNA repair and cell transformation assays in human and rodent cells clearly indicate that a single ionizing radiation track, or multiple tracks if received intermittently in time, stimulate an error-free DNA repair process. That repair system increases the probability of correctly repairing either radiation-induced or spontaneous DNA damage in the exposed cells, and by cell signal processes in neighboring unexposed cells, and therefore reduces the overall risk of either radiation-induced or spontaneous transformation to malignancy. These results are inconsistent with the LNT hypothesis and argue strongly that the hypothesis should be rejected.

Having separately examined all three possible biological outcomes of radiation damage to DNA, and the overall effect on cancer formation, the effect of a low dose exposure on cancer risk in a normal cell appears quite clear from the above cellular studies. Low doses or doses delivered at low dose rate reduce rather than increase risk in normal cells, a result that contradicts the LNT hypothesis.

Animal studies

We have reported the results of two investigations in mice that examined the adapting effects of low doses on tumor risk. One examines the influence of low doses on tumor frequency, a measure of the risk of the first "initiation" step (Figure 1). Another examines the effect on tumor latency, a measure of the speed at which the subsequent multiple steps are proceeding i.e., a measure of the risk of "lost days of life."

Table III shows the results of an experiment to investigate the influence of *in vivo* β-irradiation of mouse skin on the frequency of non-malignant skin tumors, produced by exposure to a chemical carcinogen followed by exposure to a chemical tumor-promoting agent (10). The experiment showed that skin irradiation 24 h prior to treatment with a DNA damaging chemical carcinogen reduced tumor frequency by about 5-fold. This result is consistent with the cell-based studies described above. It implies that the radiation exposure stimulated an error-free DNA repair system that was able to recognize and remove much of the chemically produced DNA damage.

Table III: Protection by β-irradiation (50 cGy surface dose) against chemical initiation of skin tumors in mice.

Initiation Treatment	**Tumors per Animal**
methyl-nitro-nitroso guanidine	2.04
β-radiation	0
β-radiation + 24h + methyl-nitro-nitroso guanidine	0.39

The above experiment measured the effects of low doses on risk by measuring the frequency of DNA damage or of transformation. We have also reported the influence of low, adapting doses on tumor latency (11). That data is summarized in Table IV and shows the influence of a prior low dose exposure delivered at low dose rate (8 mGy/min) on the latency of myeloid leukemia induced in mice by a subsequent exposure to a large dose, also delivered at that low dose rate.

Table IV: Extension of latency period in mice developing acute myeloid leukemia.

Treatment	**Average Lifespan (Days)**	**Life Lost (Days)**
Control	727	0
1.0 Gy	486	241
0.1 Gy, 24h, 1.0 Gy	578	149

The table shows that the leukemia latent period was significantly extended by the prior exposure, such that the loss of lifespan was reduced by about 40%. Interestingly, the frequency of leukemia in this experiment was unchanged. These two animal experiments show that the risk of both tumor frequency and latency can be influenced by a low dose. While both results indicate a net protective effect, the experiments suggest that the nature of the outcome may be influenced by the specific nature of the cancer risk.

Uncertainties

The data describing the responses of normal cells and normal animals to low doses of low LET radiation, and the influence of those responses on cancer risk, are convincing and show that low doses reduce rather than increase risk. On the other hand, the influence of genetics and genetic variation in individuals, as well as the response to high LET radiation is less clear.

One concern is whether low doses produce the same protective effect in individuals who are cancer prone for genetic reasons. We have recently examined this question in cancer prone mice that are heterozygous for the gene p53. This gene controls the apoptotic process and a deficiency in that process leads to cancer at about half way through a normal mouse life span. We showed that a single 10 mGy dose, delivered at a low dose rate 24h before a large high dose rate exposure extended latency for all malignant tumors in these cancer prone mice (12). This protective effect was qualitatively the same as that described above for myeloid leukemia in genetically normal mice, indicating that these genetically deficient, cancer prone mice were also protected by a low dose exposure.

Another area of uncertainty is the magnitude of variation in response to low doses, which exists in the human population that we assume is genetically normal. In the human apoptosis data quoted above, we described a low dose as causing about a 20% increase in apoptosis in lymphocytes

subsequently exposed to a high dose of radiation, and therefore reducing the risk. However, the 26 normal individuals whose lymphocytes showed this response clearly segregated into two groups. Lymphocytes from 18 individuals showed a 27.5 ± 5.7 % increase while lymphocytes from 8 other individuals averaged only 7.0 ± 3.0 % increase (8). If this difference is representative of genetic variation in the population, then the extent of the risk reduction from a low dose exposure may also be variable.

Uncertainty also exists about the effect of low doses of high LET radiation. However, it is important to recognize that the lowest possible cellular dose, i.e., that from a single track, is of the order of tens of cGy, about two orders of magnitude higher than that from a single track of low LET radiation exposure. Recent published data suggest that a single alpha track can also induce so called "bystander" effects, changes in gene activity or even DNA damage in cells adjacent to the cell actually receiving the radiation track. In addition to the bystander effects described for DNA damage and cancer formation described above, we have previously reported similar responses to low LET radiation where human lymphocytes exposed to low doses of gamma radiation, such that not all cells were traversed by a radiation track, secreted a factor that caused gene activation in other unexposed cells (13). Whether 'bystander" effects in cells which participate in immune function increase or decrease risk is unknown. However, in an animal study examining the risk of lung carcinogenesis from inhaled uranium ore dust, an alpha emitter, we observed that the frequency of lung tumors was not related to lung dose but was instead directly proportional to dose rate (14). This result implies that the risk of lung cancer from this high LET exposure was determined only by the rate of DNA repair, a process seen above to be inducible by low doses.

CONCLUSIONS

None of the predictions of the LNT hypothesis, as it applies to cancer risk from low or chronic doses of low LET radiation, are supported by the above data in human or rodent cells. The data in animals also indicates that the observed responses are not consistent with the hypothesis. The protective responses observed in mammalian cells and in animals are consistent with those seen in lower eukaryotes, including yeast, indicating that they are evolutionarily conserved (15) and lending credence to the idea that such responses are the normal and expected consequences of low dose exposures.

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